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Kathleen T. Rajkowski
United States Department of Agriculture

Steven E. Niebuhr
Iowa State University, sniebuhr@iastate.edu

James S. Dickson
Iowa State University, jdickson@iastate.edu

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Research Note

Effect of Gamma or Beta Radiation on *Salmonella* DT 104 in Ground Pork†

KATHLEEN T. RAJKOWSKI,[1]* STEVEN E. NIEBUHR,[2] AND JAMES DICKSON[2]

[1]*Food Safety Intervention Technologies Research Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038; and* [2]*Department of Animal Science, 215F Meats Laboratory, Iowa State University, Ames, Iowa 50011, USA*

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ABSTRACT

Mixtures of six *Salmonella* Typhimurium DT 104 strains were inoculated into three ground pork products to determine the effect of fat content on the radiation resistance of *Salmonella* DT 104. The ground pork products were 90% lean, 50:50 fat:lean, and 100% fat. Inoculated products were irradiated using a gamma radiation source in a self-contained ^{137}Cesium irradiator or a 10 MeV accelerator producing electrons (e-beam). The radiation D_{10}-values (dose required for a 90% inactivation of viable CFU) for *Salmonella* DT 104 inoculated into 90% lean ground pork, 50:50 fat/lean ground pork, and 100% pork fat and subjected to beta radiation were 0.42 kGy, 0.43 kGy, and 0.43 kGy, respectively. The corresponding radiation D_{10}-values for *Salmonella* DT 104 subject to gamma radiation were 0.56, 0.62, and 0.62 kGy, respectively. There was no statistical significant difference ($P = 0.3$) in radiation D_{10}-values for *Salmonella* in the three products subject to either radiation treatment. Therefore, fat content had no effect. There was a significant difference ($P = 0.001$) between the radiation D_{10}-values obtained with the two radiation sources. The radiation D_{10}-values were within the reported range for irradiation destruction of *Salmonella* contaminated raw meat products.

Salmonellosis is a leading cause of foodborne illness worldwide. The bacterial infection can be caused by any of over 2,000 serotypes of *Salmonella.* There is now concern over salmonellae that are resistant to multiple antibiotics. The antibiotic resistant strains of *Salmonella* Typhimurium isolated from humans were identified first in England and Wales *(8)* and were designated DT 104 in 1984. This strain is now the second most prevalent *Salmonella* isolated from humans in the UK *(9). Salmonella* Typhimurium DT 104 has been detected in several other countries including Canada, United States, Germany, France, Austria, and Denmark *(8).* These strains of *Salmonella* have developed resistance to multiple antibiotics including ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline (R-type ACSSuT), and more recently to trimethoprim (R-type ACSSuTTm) and ciprofloxacin (R-type ACSSuTCp) *(9).* The results of the national survey (1994–1995) in the United States reported that some human clinical isolates of salmonellaes were also quinolone-resistant *(7).*

Results of reported *Salmonella* serotypes associated with foodborne illness isolated from foods in the United States indicate that *Salmonella* Typhimurium was the second most common serotype *(8).* The proportion of *Salmonella* Typhimurium antibiotic-resistant type strains increased from 9% to 33% in the United States between 1990 and 1996 *(9).*

Salmonella serotypes have also been isolated from feces of pigs, asymptomatic carriers, raised in multiple-site production systems *(3). Salmonella* Typhimurium var. Copenhagen and/or *Salmonella* Typhimurium (76%) have been isolated from market-age pigs *(2).* Gebreyes et al. *(4, 5)* examined the antibiotic-resistant strain isolated from pig farms, transport trucks and pigs after slaughter and reported that *Salmonella* Typhimurium var. Copenhagen and *Salmonella* Typhimurium were the most common serovars that were multidrug resistant. In their 3-year study, Gebreyes et al. *(6)* stated that the two most important patterns (Ax-ACSSuT and AKSSut) and phage types (DT 104 and DT 193), which are significant public health types, constituted 66% of the *Salmonella* Typhimurium isolates. They concluded that commercial production systems for hogs may pose a risk of being a reservoir for resistant *Salmonella (6).* Control and intervention methods are being explored. But since animals, including hogs, are the asymptomatic carriers of *Salmonella,* interventions have not been fully successful. One carrier reintroduced onto a *Salmonella*-free farm (environment) can re-contaminate the whole herd *(8).*

This antibiotic-resistant pathogen was isolated from ground meat, including pork *(14)* and is considered an emerging food safety risk. Food irradiation may be an effective intervention in reducing the risk of foodborne illness associated with *Salmonella* Typhimurium DT 104 in

* Author for correspondence. Tel: 215-233-6440; Fax: 215-233-6406; E-mail: krajkowski@errc.ars.usda.gov.

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ground pork products. More information is needed concerning the effect of irradiation on *Salmonella* DT 104 inactivation.

A study of different sources of ionizing radiation, a gamma and a beta (e-beam) source, on *Salmonella* DT 104 was designed to compare how sensitive these strains are to irradiation when suspended in ground pork and whether there is a significant difference of effect between the types of irradiation sources. The effect of irradiation on DT 104 strains between different fat/lean ratios of ground pork was also examined.

MATERIALS AND METHODS

A pilot run using the linear accelerator was conducted to observe proposed doses of 0, 0.2, 0.6, 1.0, 1.4, 1.8, 2.2, 2.6, and 3.0 kGy. After the pilot run, the doses were adjusted to compensate for overlaps. Dose treatments for three replicate experiments using both the linear accelerator and gamma irradiator were: 0, 0.2, 0.6, 1.0, 1.4, 1.8, 2.4, 2.8, and 3.4 kGy. At both facilities, dosimetry was performed with alanine pellets and analyzed by using an electron paramagnetic resonance analyzer (EMS 104 EPR; Brucker Instruments Inc., Billerica, Mass.).

Bacterial species. Six strains of *Salmonella* Typhimurium DT104 were selected for the study of the effect of irradiation to obtain inactivation values. The strains came from clinical or human sources. The strains were of serotypes 104B (H2662—Hawaii and H3728—Arizona), 104 (H3380—California and H3402—Washington), and DT104 (#10 and #11). The strains were originally obtained from either Peggy Hayes (Center for Disease Control, Atlanta, Ga.) or Tom Humphrey (Public Health Laboratory Service, London, UK) and subsequently provided by Kathleen T. Rajkowski of the ARS-ERRC.

Preparation of culture. The cultures were prepared using the same procedure at both research facilities. The cultures were stored refrigerated (4 ± 1°C) until used. Three days before processing, the six isolates were inoculated into tryptic soy broth (TBS; Difco, Becton Dickinson, Sparks, Md.) and incubated overnight (18 h) at 37°C for use in preparing the inoculum. Two days prior to irradiation treatment, two 500 ml flasks containing 250 ml sterile TSB were inoculated with 1 ml of the prepared overnight DT 104 cultures and placed in a Lab Line shaker incubator model 4628CCGM or Obital shaker (Lab Line Instruments Inc., Melrose Park, Ill.) at 37°C, rotating at 120 rpm for 18 to 20 h, which represent a culture in log phase. Following incubation, the cultures were held in a refrigerator (4°C) until the inoculum was prepared by mixing equal portions of the six strains.

Preparation of product and inoculation. The strains were tested in three types of ground pork with fat levels mimicking typical meat products and consisting of the following fat:lean ratios; 10%:90% designated as L, 50%:50% designated as F/L, and greater than 90% fat:less than 10% lean designated as F. The ground pork was prepared according to the following procedure at Iowa State University (ISU) and split between the two research groups for inoculation and testing.

The pork trim was provided by the ISU Meats Laboratory where 15 lb of each product L, F/L and F were prepared. The trim was mixed according to the designated ratios and frozen in the −70°C blast freezer and stored frozen until the three products were prepared. Ground pork of the L and F/L were prepared by grinding and mixing each product twice, first through a half-inch plate and then regrinding through an eighth-inch plate, in a BIRO grinder (model 822, BIRO Inc., Marblehead, Ohio). The F product was ground only once through a half-inch plate. The L and F/L products were then made into patties in an automated patty machine (model 54, Hollymatic Inc., Countryside, Ill.). Lean patties weighed approximately 120-g and fat/lean patties weighed approximately 115 g. The F product was divided into 120-g preweighed portions and made into patties using the manual patty press (Koch Supplies Inc., Kansas City, Mo.). Paper dividers were placed between each patty. The products were then vacuum packaged on a Multivac AG 800 (Mutivac, Wolfertschwenden, Germany), frozen to −70°C, and stored. At a later date the vacuum-packaged products were then sterilized frozen by radiation with a target dose of 30 kGy. The sterilized products were stored frozen until used. A portion of these patties were sent frozen to the Agricultural Research Service—Eastern Regional Research Center (ARS) for processing at the Cesium facility.

Two days prior to irradiation treatment, the products were removed from the freezer and thawed in a refrigerator (4.0 ± 1°C). On the day of inoculation, 390 g of each sterile product (L, F/L, and F) was placed into stomacher bags (Seward, London, UK) and 39 ml of the inoculum mixture was added to each sample. To assure adequate dispersion of the cells, each bag was stomached for 1 min then manually mixed by hand for 30 s, and stomached for 30 s longer. For processing by the accelerator beam, 25 g portions were aseptically removed and placed into sterile 60 × 15 mm petri dishes (Fisher Scientific, Chicago, Ill.) for each of the nine doses. The outside of the dishes was then sanitized by either a Lysol/alcohol solution or a hyper-chlorite solution to remove any bacterial contamination for handling outside the microbiology laboratory. For processing by gamma irradiation, 25-g portions were aseptically removed, placed into stomacher bags, and taped shut. After weighing, all prepared samples were stored refrigerated (4.0 ± 1°C) overnight and irradiated at the refrigerated temperature (4.0 ± 1°C).

The following morning the dishes were placed in a Thermos cooler to maintain the temperature during transport and irradiated at the Iowa State University's Linear Accelerator Facility by a MeV CIRCE III Linear Electron Accelerator (MeV Industries, Buc, France). Following the same procedure, the samples were irradiated at ARS using a ^{137}Cesium self-contained gamma radiation source (Lockheed Georgia Company) with a strength of approximately 109,159 Ci and a dose rate of 0.105 kGy min^{-1}. The dose rate was established using National Institute of Standards and Technology (Gaithersburg, Md.) alanine transfer dosimeters. Variations in doses were minimized by placement within a uniform area of the radiation field. The samples were either returned to refrigeration or placed on ice for plating.

Enumeration. The culture inoculum was serially diluted and plated on TSA the day the samples were inoculated to obtain an initial count. After e-beam treatment the samples including the control (N_0) were aseptically removed from the dishes, placed in stomacher bags and diluted to 10^{-1} in 0.1% peptone water by using the ASAP diluter (Spiral Systems Inc., Cincinnati, Ohio). The samples were then stomached using a Stomacher Lab Blender 400 (Seward Medical, London, UK) for 2 min. After gamma treatment the stomacher bags were aseptically opened and the sample diluted to 10^{-1} and stomached as described above. Serial dilutions were made in 0.1% peptone depending on the radiation dose applied. All prepared samples were retained on ice to inhibit growth while plating. Both a Spiral Plater model D (Spiral Systems Inc., Cincinnati, Ohio) and a Whitley Automatic Spiral Plater (Don Whiteley Scientific Limited, West Yorkshire, UK) were used for plating on TSA and duplicate plates were made for each dilution

TABLE 1. D_{10}-*value for* Salmonella *Typhimurium DT 104 mixture inoculated onto ground pork*

Meat type	D_{10}-value (kGy)			
	e-Beam	R^2	Gamma	R^2
Lean	0.42 ± 0.04[a]	>0.98	0.56 ± 0.05[a]	>0.97
F/L	0.43 ± 0.02[a]	>0.99	0.62 ± 0.06[a]	>0.95
Fat	0.43 ± 0.03[a]	>0.98	0.62 ± 0.06[a]	>0.98
Average	0.43 ± 0.03[b]	>0.98	0.60 ± 0.06[b]	>0.95

[a] No significant difference between meat products at $P = 0.3$ based on fat content.
[b] Significant difference between treatment at $P = 0.0001$ based on irradiation source.

plated. At ISU enumeration was accomplished by utilizing the Protos colony counter (Synoptics Ltd., Cambridge, UK) and at ARS the plates were counted using a spiral laser colony scanner (Model 500A, Spiral Biotech).

Statistical analysis. The radiation D_{10}-values were obtained using Excel's linear regression analysis. To avoid any shoulder effects the zero-dose values were excluded from the calculation and a minimum of five values in the linear portion of the inactivation curves were used. The results of three independent replicates for each irradiation process were pooled and compared. Statistical analysis was conducted using SAS (V6.12) using the general linear model (GLM) procedure. The comparison of the radiation D_{10}-values of gamma and e-beam was analyzed using the Tukey (analysis of variance) of the SAS program.

RESULTS AND DISCUSSION

The radiation D_{10}-values for *Salmonella* DT 104 pathogen in log phase was inoculated into three ground pork products after radiation by the e-beam and gamma process are compared in Table 1. There was no significant difference in radiation D_{10}-values between the three different ground meat samples for the individual radiation process, therefore fat content of the pork products had no affect. There was, however, a significant difference in radiation D_{10}-value between the two processes as seen in the difference in slopes of the generated curves (Fig. 1). The overall average radiation D_{10}-values for the e-beam and gamma process were 0.43 ± 0.03 kGy and 0.60 ± 0.06 kGy, respectively, and are significantly different at $P = 0.0001$ as determined by Tukey's analysis of variance.

There are no previous reported studies on the radiation survival of *Salmonella* DT 104 in pork products. There is literature data on the effect of radiation of *Salmonella* Typhimurium inoculated on beef and poultry. In their study Chung et al. *(1)* reported that low levels of radiation (3 kGy) were effective in inactivating *Salmonella* Typhimurium ATCC 13311 inoculated on beef. Thayer et al. *(13)* reported a *D*-value of 0.53 kGy for *Salmonella* Typhimurium ATCC 14028 inoculated on poultry and radiated in air. The value reported by Thayer et al. *(13)* compared well with the radiation D_{10}-value after gamma radiation observed in this study for the DT 104. In their reported studies where *Salmonella* Typhimurium (14028) was part of the inoculum cocktail, Thayer et al. reported a radiation D_{10}-value of 0.51 ± 0.03 kGy on pork *(12)* and a range in values of 0.50 to 0.55 kGy for exotic meats *(11)*.

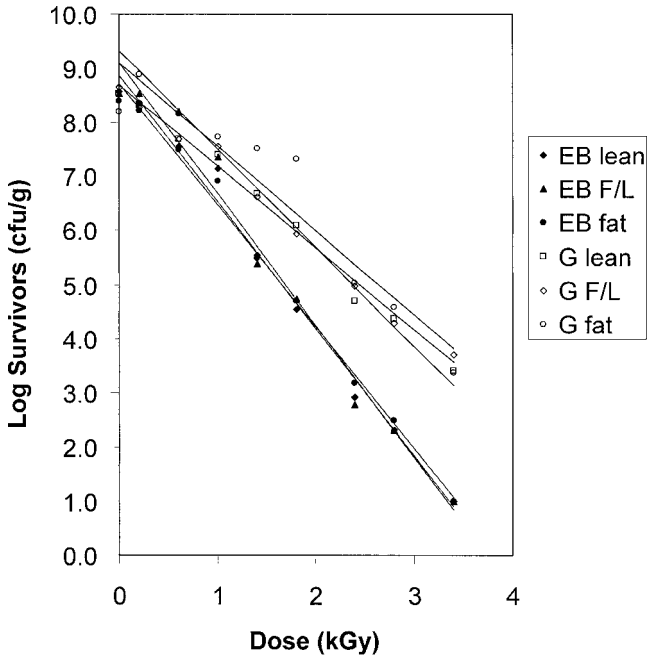

FIGURE 1. *Comparison of the radiation inactivation curves for a mixture of* Salmonella *Typhimurium DT 104 isolates in ground pork products processed using an electron beam accelerator (closed marks) and a gamma irradiator (open marks). Each component was conducted independently three times* (n = *3).*

In this comparison study between gamma and beta irradiation, three ground pork split samples were inoculated with a mixture of DT 104 strains obtained from the same source and prepared using the same protocol. All samples were treated and maintained the same before and after irradiation, except for the packaging of the product. The meat samples (25 g) were in plastic petri dishes and placed in the e-beam accelerator for treatment, and for the gamma process the meat was placed in stomacher bags and sealed using tape. There were packaging differences of the meat products in this study. López-González et al. *(10)* determined the influence of various commercial packaging conditions on the survival of *Escherichia coli* O157:H7 by electron beam and gamma radiation. They concluded that gamma radiation resulted in higher radiation D_{10}-values as compared to electron beam and is dependent on the temperature, atmosphere, and medium in which the pathogen is irradiated. Similar results between the gamma and electron beam results were observed in this comparison study where the values for gamma radiation were significantly higher. The radiation D_{10}-value 0.43 kGy for e-beam versus 0.60 kGy for gamma results may be due to the packaging difference. Product thickness also influences the MAX/MIN ratio in commercial irradiators. In this study, the products for the e-beam treatment were evenly distributed in the plastic petri dishes (thin layer), whereas the products for gamma treatment were placed in a bag (clump). During gamma radiation treatment, the chamber was flushed with nitrogen to control temperature which removes oxygen, whereas e-beam treatment had oxygen in the chamber. The

difference in packaging material, sample geometry and atmosphere in this study did influence the radiation D_{10}-values, which also supports López-González et al. *(10)* conclusions as to resulting differences. Future comparison studies using gamma and e-beam radiation need to be conducted on products packaged under the same conditions (thickness) and in the same packaging material and irradiated under the same atmosphere conditions.

Although there is a difference between the radiation D_{10}-values obtained after using an e-beam and gamma irradiator, the values do fall within previous reported radiation D_{10}-value ranges for *Salmonella* on meat products, which may be insignificant from the viewpoint of commercial processing. Low levels of radiation by gamma or electron beam will assure the inactivation of any *Salmonella* in raw meats, including ground pork, which makes for a safer product for the consumer.

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